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REPORT**Distinct HLA associations of LGI1 and CASPR2-antibody diseases**

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The recent biochemical distinction between antibodies against leucine-rich, glioma-inactivated-1 (LGI1), contactin-associated protein-2 (CASPR2) and intracellular epitopes of voltage-gated potassium-channels (VGKCs) demands aetiological explanations. Given established associations between human leucocyte antigen (HLA) alleles and adverse drug reactions, and our clinical observation of frequent adverse drug reactions in patients with LGI1 antibodies, we compared HLA alleles between healthy controls ($n = 5553$) and 111 Caucasian patients with VGKC-complex autoantibodies. In patients with LGI1 antibodies ($n = 68$), HLA-DRB1*07:01 was strongly represented [odds ratio = 27.6 (95% confidence interval 12.9–72.2), $P = 4.1 \times 10^{-26}$]. In contrast, patients with CASPR2 antibodies ($n = 31$) showed over-representation of HLA-DRB1*11:01 [odds ratio = 9.4 (95% confidence interval 4.6–19.3), $P = 5.7 \times 10^{-6}$]. Other allelic associations for patients with LGI1 antibodies reflected linkage, and significant haplotypic associations included HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02, by comparison to DRB1*11:01-DQA1*05:01-DQB1*03:01 in CASPR2-antibody patients. Conditional analysis in LGI1-antibody patients resolved further independent class I and II associations. By comparison, patients with both LGI1 and CASPR2 antibodies ($n = 3$) carried yet another complement of HLA variants, and patients with intracellular VGKC antibodies ($n = 9$) lacked significant HLA associations. Within LGI1- or CASPR2-antibody patients, HLA associations did not correlate with clinical features. *In silico* predictions identified unique CASPR2- and LGI1-derived peptides potentially presented by the respective over-represented HLA molecules. These highly significant HLA associations dichotomize the underlying immunology in patients with LGI1 or CASPR2 antibodies, and inform T cell specificities and cellular interactions at disease initiation.

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Abbreviations: HLA = human leucocyte antigen; VGKC = voltage-gated potassium channel

Introduction

The discovery of autoantibodies against leucine-rich, glioma-inactivated 1 (LGI1), contactin-associated protein 2 (CASPR2) (Irani *et al.*, 2010; Lai *et al.*, 2010) and, more recently, intracellular epitopes of voltage-gated potassium channels (VGKCs) (Lang *et al.*, 2017), have redefined the immunology of the VGKC-complex (Thieben *et al.*, 2004; Vincent *et al.*, 2004). Patient stratification by these antigenic targets has shown that the ‘double-negative’ VGKC-complex antibodies, those without LGI1 or CASPR2 reactivities, are observed across all ages, in healthy controls and in a variety of syndromes, many of which are not immune-mediated (Graus and Gorman, 2016; van Sonderen *et al.*, 2016; Lang *et al.*, 2017). In contrast, patients with LGI1 or CASPR2 antibodies often have clinically-indistinguishable late-onset forms of limbic encephalitis and neuromyotonia with associated dysautonomia, sleep disturbances, pain and seizures (Irani *et al.*, 2010; Lai *et al.*, 2010; Klein *et al.*, 2013; Gadoth *et al.*, 2017). While these features occur at different rates in LGI1-versus CASPR2-antibody cohorts, only faciobrachial dystonic seizures (FBDS) robustly predict LGI1 reactivity (Irani *et al.*, 2011; Gadoth *et al.*, 2017; Thompson *et al.*, 2018). Furthermore, these two autoantibodies are both often of the IgG4 subclass and frequently co-exist in patients with the ultra-rare Morvan’s syndrome (Irani *et al.*, 2012; Ariño *et al.*, 2016). The striking overlaps of these rare neurological features and autoantibodies, and the frequent co-expression of their antigenic targets within mammalian CNS-membrane complexes (Irani *et al.*, 2010; Binks *et al.*, 2018), suggest they are involved in autoimmunization. Indeed, this has been reported in abattoir workers with autoantibodies against VGKC-complexes and, less so, CASPR2 (Meeusen *et al.*, 2012). The nature of the available complexes, antigen presentation mechanisms and the available T cell repertoires are likely to determine which antigen dominates the ensuing T–B cell response. If so, human leucocyte antigen (HLA) variants, intimately related to antigen presentation, may play critical roles in distinguishing the aetiology of these syndromes.

Previously, high rates of adverse drug reactions were observed in patients with LGI1 antibodies, typically secondary to antiepileptic drugs (AEDs) and, less so, corticosteroids (Irani *et al.*, 2011, 2013; Thompson *et al.*, 2018). As HLA variants have been implicated in several adverse drug reactions, including those associated with AEDs and

immunosuppressants (McCormack *et al.*, 2011; Yip *et al.*, 2015), and have essential antigen-presenting functions (Trowsdale and Knight, 2013), we hypothesized that HLA associations existed in patients with LGI1 antibodies. Indeed, recently, HLA-DRB1*07:01, HLA-DQB1*02:02 and HLA-DRB4 were found to be present in varying proportions of patients with LGI1 antibodies in two cohorts totalling 40 patients, from Korea and the Netherlands (Kim *et al.*, 2017; van Sonderen *et al.*, 2017).

To extend these early observations, and given the hypothesis that the VGKC complex may be the initiating immunizing agent, we sought to compare and contrast HLA-associations in a sizeable cohort of clinically well-characterized patients with antibodies against LGI1, CASPR2, both LGI1 and CASPR2, and VGKCs, and *in silico* to identify peptides that may be presented by these HLA molecules.

Materials and methods

Patients

One hundred and eleven Caucasian patients were identified from previous studies ($n = 51$) (Irani *et al.*, 2011, 2013; Lang *et al.*, 2017), referrals to the Oxford Autoimmune Neurology Group ($n = 46$) or from the Autoimmune Encephalopathy Clinic, University of California San Francisco ($n = 14$). These patients had serum antibodies against LGI1 only ($n = 68$), CASPR2 only ($n = 31$), both LGI1 and CASPR2 ($n = 3$) or intracellular aspects of VGKCs ($n = 9$), as determined by previously described antigen-specific cell-based assays (Irani *et al.*, 2010; Lang *et al.*, 2017). Clinical phenotypes, including information relating to past medical history and adverse drug reactions (Table 1), were evaluated via direct patient and relative interviews and case-note reviews. All patients provided written informed consent (REC16/YH/0013 or the IRB 10-04905 approvals).

Genotyping, HLA imputation, verification and multi-locus haplotype-block construction

The Infinium Global Screening Array-24 + v1.0 BeadChip with Illumina Infinium HTS custom markers were used for genotyping. We proceeded to impute HLA alleles using SNP2HLA at eight classical loci (HLA-A, HLA-B, HLA-C, HLA-DPA, HLA-DPB, HLA-DQA1, HLA-DQB1 and HLA-DRB1) at two-field resolution (Jia *et al.*, 2013; Neville *et al.*, 2017).

Table 1 Clinical features of patients with antibodies to VGKC complex proteins: LGII, CASPR2, both LGII and CASPR2 or intracellular aspects of VGKCs

| | LGII | CASPR2 | LGII and CASPR2 | VGKC | LGII versus CASPR2 (P-value)* |
|---|---------------|--------------|-----------------|------------|-------------------------------|
| Number of patients | 68 | 31 | 3 | 9 | ND |
| Median age at onset age (range) | 63 (41–85) | 68 (19–82) | 56 (52–65) | 43 (33–71) | ND |
| Female (%) | 20 (29) | 2 (6) | 1 (33) | 4 (44) | ND |
| Clinical syndrome (%) | | | | | |
| Epilepsy | 8 (12) | 2 (6) | 0 (0) | 4 (44) | ND |
| Encephalitis | 58 (85) | 18 (58) | 1 (33) | 2 (22) | ND |
| Morvan's | 1 (2) | 3 (10) | 2 (67) | 0 (0) | ND |
| Isolated neuromyotonia | 0 (0) | 2 (6) | 0 (0) | 0 (0) | ND |
| Other ^a | 1 (2) | 6 (19) | 0 (0) | 3 (33) | ND |
| Clinical features (%) | | | | | |
| Any seizure | 66 (97) | 25 (81) | 2 (67) | 7 (78) | 0.01 |
| Faciobrachial dystonic seizures | 47 (69) | 0 (0) | 1 (33) | 0 (0) | <0.0001 |
| Generalized seizure | 26 (38) | 10 (32) | 2 (67) | 6 (67) | NS |
| Amnesia | 58 (85) | 23 (74) | 3 (100) | 5 (56) | NS |
| Neuromyotonia | 1 (2) | 8 (26) | 2 (67) | 0 (0) | 0.0003 |
| Neuropathic pain | 5 (7) | 14 (45) | 2 (67) | 0 (0) | <0.0001 |
| Autoimmune features (%) | | | | | |
| Other autoimmune disease ^b | 19 (28) | 7 (23) | 0 (0) | 0 (0) | NS |
| Atopy | 8 (12) | 2 (6) | 2 (67) | 1 (11) | NS |
| Adverse effects of corticosteroids ^c | 32 (47) | 5 (16) | 0 (0) | 1 (11) | 0.004 |
| Drug rash | 24 (35) | 1 (3) | 0 (0) | 0 (0) | 0.0004 |
| Other features | | | | | |
| Mean change in mRS (range) | 1.6 (−3 to 4) | 1.5 (0 to 4) | 1.7 (0 to 3) | 1 (0 to 2) | ND |
| Tumour (%) ^d | 9 (13) | 4 (13) | 2 (67) | 1 (11) | ND |

Live cell-based assays were used for LGII and CASPR2 antibody determination (Irani *et al.*, 2010), and fixed assays to detect antibodies against the intracellular aspects of VGKCs (Lang *et al.*, 2017).

^aOther diagnoses included movement disorders ($n = 4$, CASPR2, generalized chorea, hemifacial spasm, cervical dystonia and cerebellar ataxia), psychogenic amnesia ($n = 2$, VGKC antibodies), widespread non-neuropathic pain ($n = 1$, VGKC antibodies), axonal neuropathy ($n = 1$, CASPR2), psychosis ($n = 1$, CASPR2) and stroke ($n = 1$ with LGII antibodies). Two patients with antibodies against intracellular VGKC epitopes had epilepsy secondary to structural lesions.

^bAutoimmune diseases in LGII-antibody patients [$n = 19$: diabetes ($n = 1$), heparin-induced thrombocytopenia ($n = 1$), hyper- and hypothyroidism and Hashimoto's thyroiditis ($n = 8$), multiple sclerosis ($n = 1$), myasthenia gravis ($n = 1$), neuromyelitis optica ($n = 1$), optic neuritis ($n = 1$), pernicious anaemia ($n = 1$), psoriasis ($n = 6$), Raynaud's disease ($n = 1$), and ulcerative colitis ($n = 1$)] and CASPR2-antibody patients [$n = 7$: congenital adrenal hyperplasia, hypothyroidism, pernicious anaemia, pemphigus, polymyalgia rheumatica, psoriasis and Raynaud's disease (all $n = 1$)].

^cCorticosteroid-related complications, sometimes multiple, in LGII-antibody patients [$n = 32$: marked weight gain ($n = 12$), behavioural disturbance ($n = 5$) and diabetes ($n = 5$), or worsened diabetes ($n = 1$), insomnia ($n = 4$), fracture ($n = 3$), myopathy or muscle weakness ($n = 3$), skin thinning/easy bruising ($n = 3$), mania/hypomania ($n = 2$), poor wound healing or abscess ($n = 2$), ophthalmic infections ($n = 2$; keratitis and ophthalmic shingles), perforated abdominal viscus ($n = 2$), and one each of: avascular necrosis of the hip (AVN), cerebral venous sinus thrombosis, high INR and steroid-induced psychosis] and in CASPR2-antibody patients [$n = 5$: marked weight gain ($n = 1$), rash ($n = 2$), striae/thin skin/bruising ($n = 2$), and hallucinations ($n = 1$)].

^dTumours in LGII-antibody patients ($n = 9$) were: basal cell carcinoma ($n = 3$), other skin – type not known ($n = 2$), bladder ($n = 1$), breast ($n = 1$), prostate ($n = 1$), dysplastic colonic polyp ($n = 1$) and in 4 CASPR2-antibody patients were: pancreatic ($n = 1$), prostate ($n = 2$), thymic cyst ($n = 1$).

NS = not significant; ND = not done; mRS = modified Rankin scale (as Thompson *et al.*, 2018).

*Statistical comparisons with Fisher's exact test throughout.

To complement this, DRB1, DRB4 and DQ alleles underwent intermediate-resolution HLA-typing using PCR-sequence-specific primers (SSP), updated from Bunce *et al.* (1995). PCR-SSP defined the first-field plus a string of second-field possibilities: the highest frequency allele in Caucasians was considered most likely. For all discordant data, the PCR-SSP first-field was accepted as the final result. HLA alleles from 5553 Caucasian healthy controls (from Oxford Biobank) were available from imputation using the same platforms, and confirmed in 70 individuals within the same laboratory by PCR-SSP (Neville *et al.*, 2017). Probable haplotype blocks were calculated on the basis of a Bayesian algorithm using PHASE V2 software with 10 000 iterations for three haplotype blocks: HLA-C-B, HLA-DRB1-DQA1-DQB1, and HLA-

DPA1-DPB1 (Stephens *et al.*, 2001; Stephens and Donnelly, 2003). Further details on genotyping and imputation are provided in the Supplementary material.

Statistical analyses

For each antibody group, Fisher's exact test (two-tailed) was used to compare the HLA allele and haplotype carrier frequencies between patients and the healthy control dataset. Hochberg's method was used to correct for multiple comparisons. Corrected P -values < 0.05 were considered significant, and are presented. Odds ratios (ORs) were calculated using the median-unbiased estimation method.

HLA binding predictions

The NetMHCIIpan 3.1 server model based on artificial neural networks (Andreatta *et al.*, 2015) evaluated HLA haplotype binding affinities for 15-amino acid-long consecutive overlapping peptides from full-length LGI1 and CASPR2 sequences (UniProt accession numbers O95970 and Q9UHC6, respectively). Predicted peptide affinities (nM) were compared to 200 000 random peptides of the same length to generate rank values: this measure is less susceptible to the intrinsic capacity of some HLA alleles to generate high-affinity predictions, and rank values (%) <3 were considered strong binders. As expected, consecutive 15-mer peptides with high rank values often shared a core sequence.

Results

Clinical differences between patients stratified by VGKC-complex autoantibody targets

Table 1 summarizes the clinical features of the 111 patients, subgrouped by their autoantibody specificities. In agreement with previous studies, onset ages were typically around 60 years, and patients with LGI1 or CASPR2 antibodies most frequently had encephalitis or epilepsy. FBDS were exclusive to patients with LGI1 antibodies ($P < 0.0001$) who had more seizures ($P = 0.01$) than patients with CASPR2 antibodies, where peripheral nerve features of neuromyotonia ($P = 0.0003$) and neuropathic pain ($P < 0.0001$) were preferentially associated. As expected, the nine patients with antibodies to intracellular VGKC epitopes had heterogeneous, often non-immune, clinical syndromes. By contrast, likely non-immune syndromes were noted in only one patient with LGI1 antibodies (stroke) and in four with CASPR2 antibodies (axonal neuropathy, cervical dystonia, hemifacial spasm and psychosis).

Of greater relevance to a HLA study, patients with LGI1 or CASPR2 antibodies often had co-existent autoimmune conditions (28% and 23%, respectively), including Hashimoto's thyroiditis ($n = 8$), psoriasis ($n = 7$) and pernicious anaemia ($n = 2$). Moreover, the LGI1-antibody cohort was distinctive for a 47% rate of adverse drug reactions from corticosteroids ($P = 0.004$; 16% with CASPR2 antibodies) and a significantly higher rate of drug-induced rashes (35% versus 3% in CASPR2, $P = 0.0004$). The reported rashes were secondary to AEDs [$n = 13$: including carbamazepine ($n = 6$), phenytoin ($n = 4$), lamotrigine ($n = 2$) and valproate ($n = 1$)], antibiotics [$n = 6$: penicillins ($n = 5$) and metronidazole ($n = 1$)] and immunosuppressants [$n = 5$: azathioprine ($n = 2$), corticosteroids ($n = 2$) and methotrexate ($n = 1$)]. Thus, the LGI1- and CASPR2-antibody groups displayed differing clinical autoimmune features suggesting divergent immunogenetic pathways.

Patients with LGI1 or CASPR2 antibodies have strong and distinct HLA allelic profiles

We proceeded to analyse HLA associations, as summarized in Fig. 1 and Table 2. Consistent with previous smaller reports (Kim *et al.*, 2017; van Sonderen *et al.*, 2017), almost all LGI1-antibody-positive patients carried HLA-DRB1*07:01 (91%, compared to 26% in healthy controls) [OR 27.6 (95% confidence interval, CI 12.9–72.2), $P = 4.1 \times 10^{-26}$]. Further, 13% (9/68) were homozygous for DRB1*07:01, compared to 2% (115/5553) healthy controls [OR 7.3 (95% CI 3.3–14.4), $P = 3 \times 10^{-4}$]. Alleles recognized to be part of haplotypes involving HLA-DRB1*07:01 (González-Galarza *et al.*, 2015) were over-represented, namely HLA-DQA1*02:01, HLA-DQB1*02:02, HLA-DQB1*03:03 and HLA-DPB1*11:01. Additionally, associations were found with two HLA class I alleles, HLA-B*57:01 [OR = 3.7 (95% CI 2.0–6.5); $P = 0.014$] and HLA-C*06:02 [OR = 3.9 (95% CI 2.4–6.3); $P = 4.6 \times 10^{-5}$]. After conditioning on the commonest allele, HLA-DRB1*07:01, two other DQ alleles reached statistical significance consistent with evidence of an independent association, HLA-DQA1*01:03 [OR = 4.4 (95% CI 2.2–8.1); $P = 4 \times 10^{-3}$] and HLA-DRB1*01:03 [OR = 14.7 (95% CI 3.6–51.5), $P = 0.04$].

In striking contrast, analysis of the CASPR2-antibody group identified a single risk allele; HLA-DRB1*11:01, which was present in 48% of CASPR2-antibody patients compared to 4% of patients with LGI1 antibodies and 9% of healthy controls [OR 9.4 (95% CI 4.6–19.3); $P = 5.7 \times 10^{-6}$]. One CASPR2-antibody patient was homozygous for HLA-DRB1*11:01. Interestingly, the four patients with non-immune conditions and CASPR2 antibodies (Table 1) did not carry HLA-DRB1*11:01, giving it a 56% (15/27) frequency in the remainder. No additional alleles were observed after conditioning on HLA-DRB1*11:01.

Intriguingly, of the three patients with co-existent CASPR2 and LGI1 antibodies, only one carried HLA-DRB1*07:01 and none carried HLA-DRB1*11:01. However, all three carried HLA-B*44:02, HLA-C*05:01, HLA-DQA1*03:01 and HLA-DQB1*03:01, a different complement of alleles to the patients with antibodies to either LGI1 or CASPR2 (Supplementary Fig. 1). There were no significant findings within the group with intracellular VGKC antibodies (Supplementary Fig. 1).

Haplotype-specific distinctions between patients with LGI1 and CASPR2 antibodies

Next, to understand the *en bloc* allelic inheritance and *in vivo* relevance of HLA combinations that may present LGI1 and CASPR2 antigens, we explored associations involving HLA haplotypes (Fig. 1B and full analysis in Supplementary Figs 2–4). We noted that HLA-DQA1*02:01, HLA-DQB1*02:02,

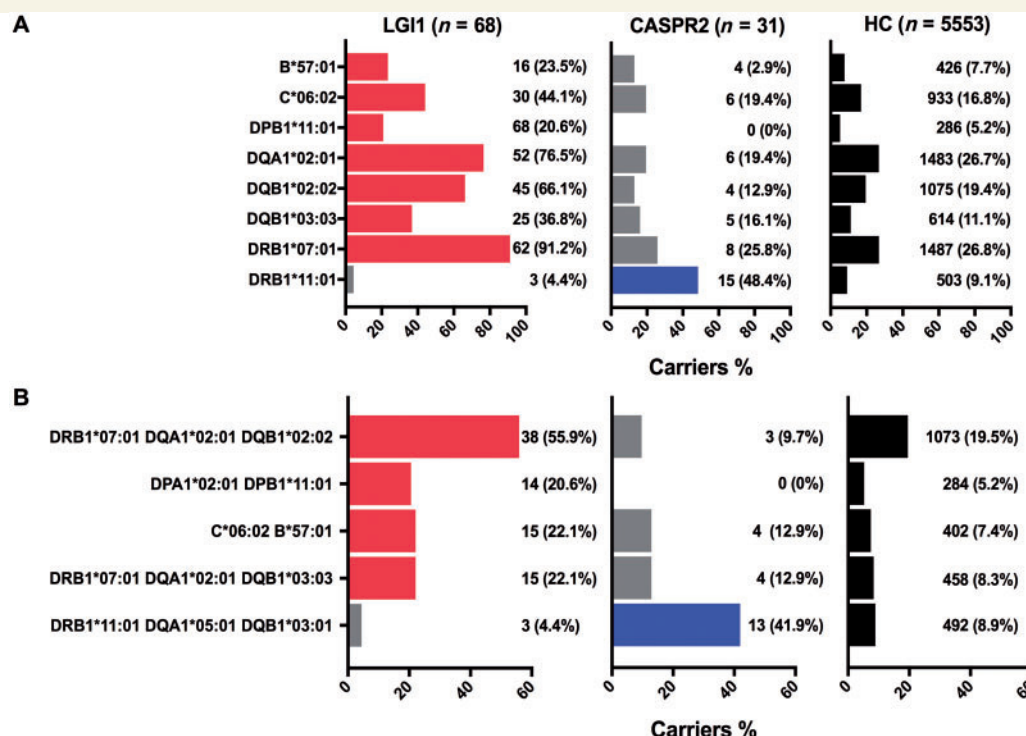


Figure 1 HLA allele and haplotype associations in patients with LGI1 and CASPR2 antibodies. Bar chart depicting allele (A) and haplotype (B) associations and their frequency in patients with antibodies to LGI1 ($n = 68$, red denotes significant associations) and CASPR2 ($n = 31$, blue denotes significant associations), together with the frequency of these alleles or haplotypes in 5553 healthy controls (black bars). HC = healthy controls.

HLA-DQB1*03:03 and HLA-DPB1*11:01 show evidence of linkage disequilibrium with HLA-DRB1*07:01 (r^2 values 0.64, 0.49, 0.13, 0.10 and D' 1, 0.95, 0.8 and 1, respectively) (Supplementary material). This was reflected in the most frequent HLA class II haplotypes found in patients with LGI1 antibodies, namely HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02 [OR = 5.2 (95% CI 3.2–8.6); $P = 2.3 \times 10^{-9}$], DRB1*07:01-DQA1*02:01-DQB1*03:03 [OR = 3.1 (95% CI 1.7–5.5); $P = 0.02$] and DPA1*02:01-DPB1*11:01 [OR = 4.8 (95% CI 2.5–8.5); $P = 3.8 \times 10^{-4}$]. In addition, LGI1-antibody status was associated with a HLA class I haplotype, HLA-C*06:02-B*57:01 [OR = 3.6 (95% CI 1.9–6.2); $P = 8.8 \times 10^{-3}$]. By contrast, only one HLA class II haplotype was associated with CASPR2 antibodies: DRB1*11:01-DQA1*05:01-DQB1*03:01 [OR = 7.4 (95% CI 3.5–15.2), $P = 5.7 \times 10^{-5}$].

Given these significant and distinct allelic and haplotypic HLA associations, for each serologically-defined group, we evaluated their value in explaining sub-phenotypes (limbic encephalitis or epilepsy; peripheral or CNS), long-term outcomes or adverse drug reactions (Supplementary Table 1), and found no significant HLA allele or haplotype associations. However, within LGI1-antibody patients, five of six patients with antibiotic-induced rashes carried HLA-B*57:01 known to associate with risk of rash to abacavir and flucloxacillin (Yip *et al.*, 2015), and four of six patients

with psoriasis harboured the psoriasis risk allele C*06:02 (Arakawa *et al.*, 2015), suggesting the extended haplotypes may explain these specific co-morbidities. Finally, from the nine LGI1- and four CASPR2-antibody patients with a tumour, there were no significant HLA differences compared to non-tumour patients (Supplementary Table 2).

DRB4 analysis

To extend a previous report (van Sonderen *et al.*, 2017), the HLA-DRB1 paralogue HLA-DRB4 was sequenced and observed to be absent or a null allele in 61% (19/31) of the CASPR2-antibody cohort and 44% (4/9) of the intracellular VGKC-antibody cohort, consistent with the healthy control frequencies. However, only 16% (11/68) of the LGI1-antibody cohort showed an absent or null HLA-DRB4 allele, and these 11 patients all carried HLA-DRB1*07:01 (Supplementary Table 3).

Predictions of HLA-binding peptides

These robust HLA class II associations strongly implicate CD4⁺ T cells in the pathogenesis of both LGI1- and CASPR2-antibody-associated diseases. To locate potentially high-affinity peptides that complex with HLA class II heterodimers, and may interact with patient T cells, *in silico*

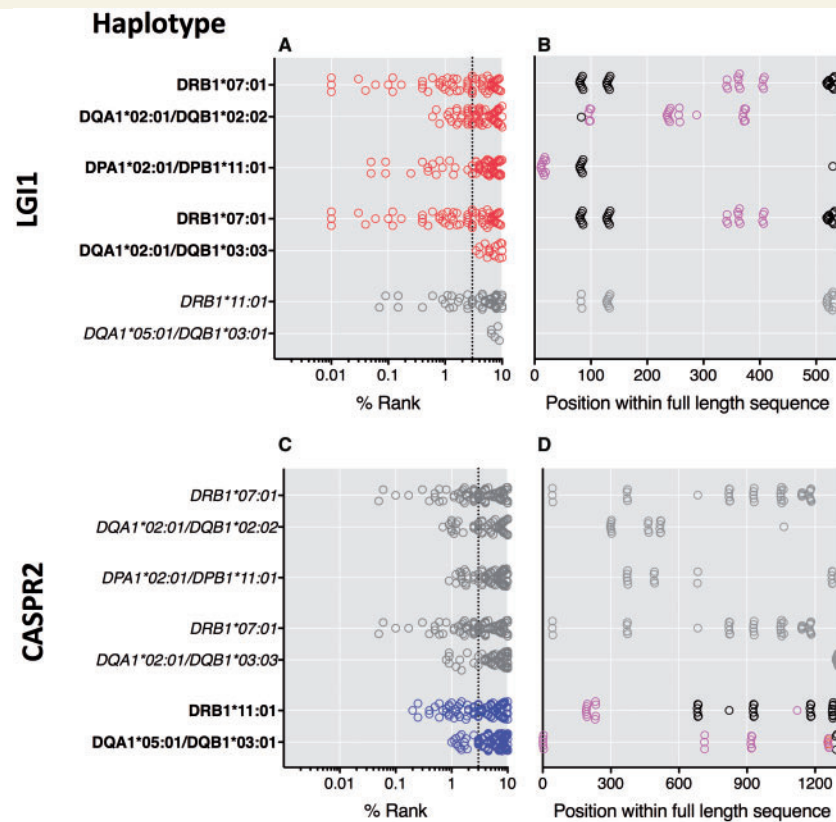


Figure 2 Peptides derived from full-length LGI1 and CASPR2 predicted to bind MHC-dimers encoded by over-represented HLA haplotypes. Rankings and position of peptides derived from full-length sequences of LGI1 (**A** and **B**) and CASPR2 (**C** and **D**). The haplotypes correspond to Fig. 1B and when in bold they relate to those observed in patients with antibodies to the corresponding protein. Red circles denote the LGI1-antibody cohort and blue the CASPR2-antibody cohort. Grey circles and italicized haplotypes relate to peptides from the other antigenic protein (i.e. CASPR2 in **A** and **B**; and LGI1 in **C** and **D**). Rank describes the predicted peptide affinities (IC_{50} , nM) by comparison to 200 000 random peptides of the same length. Dotted lines represent the 3% cut-off for peptide rank. Within **B** and **D**, circles represent the highly-ranked peptides across the full-length sequences of LGI1 or CASPR2: black circles represent peptides with some predicted promiscuity across LGI1- and CASPR2-antibody HLA variants, whereas pink circles highlight peptides that are not predicted to cross-react.

modelling was used and focused on all the class II haplotypes identified above (Fig. 2).

Overall, many peptides from both LGI1 and CASPR2 ranked highly for potential binding to several HLA-DR, HLA-DP and HLA-DQ variants (Fig. 2A and C), likely consistent with the varied intrinsic properties of different HLA molecules. Furthermore, for HLA-DRB1*07:01 and HLA-DRB1*11:01, which pair with the invariant DRA chain, and for HLA-DQA1*02:01-DQB1*02:02 heterodimers, peptide ranks showed little difference between LGI1- and CASPR2-derived peptides, suggesting a lack of antigen selectivity. Also, no highly-ranked peptides were identified to bind the LGI1-antibody-associated heterodimer HLA-DQA1*02:01-DQB1*03:03, making it an unlikely candidate molecule for LGI1 peptide presentation. By contrast, the CASPR2-antibody-associated HLA-DQA1*05:01-DQB1*03:01 heterodimer was predicted to bind some high-ranking peptides from the CASPR2 sequence only, suggesting CASPR2 specificity.

As expected for the shared core sequences between consecutive 15-mers, many highly ranked peptides were from tightly clustered locations within the full-length protein (Fig. 2B, D and Supplementary Table 4). Most peptides within these clusters showed potential to bind the HLA variants observed in both the LGI1- and CASPR2-antibody cohorts (Fig. 2B and D, black circles). This included one previously identified peptide (Kim *et al.*, 2017) and argues against its role in disease specificity. However, 9/13 LGI1-derived peptides and 7/13 from the CASPR2 sequence showed binding potential that was more restricted to the variants associated with the corresponding antibody cohort (Fig. 2B and D, pink circles). From LGI1, 4/9 core peptides were predicted to bind with high affinity (<40 nM), typically to HLA-DRB1*07:01, although interestingly the highest affinity peptide was predicted to bind HLA-DPA1*02:01-DPB1*11:01 (Supplementary Table 4). From CASPR2-derived peptides, 7/7 were predicted to bind with high affinity, distributed across the variants within the

Table 2 Significant allele (top) and haplotype (bottom) associations in patients with LGI1 (*n* = 68) or CASPR2 (*n* = 31) antibodies

| Antibody group | Allele / haplotype | OR | 95% CI lower | 95% CI upper | Fisher's exact test | Corrected (Hochberg) P-value |
|----------------|----------------------------------|------|--------------|--------------|-----------------------|------------------------------|
| LGI1 | B*57:01 | 3.7 | 2.0 | 6.5 | 5.2×10^{-5} | 1.0×10^{-2} |
| LGI1 | C*06:02 | 3.9 | 2.4 | 6.3 | 1.6×10^{-7} | 4.6×10^{-5} |
| LGI1 | DPB1*11:01 | 4.9 | 2.6 | 8.7 | 8.4×10^{-6} | 2.0×10^{-3} |
| LGI1 | DQA1*02:01 | 8.9 | 5.2 | 16.1 | 3.0×10^{-17} | 8.7×10^{-15} |
| LGI1 | DQB1*02:02 | 8.1 | 4.9 | 13.7 | 9.5×10^{-17} | 2.8×10^{-14} |
| LGI1 | DQB1*03:03 | 4.7 | 2.8 | 7.7 | 3.6×10^{-8} | 1.0×10^{-5} |
| LGI1 | DRB1*07:01 | 27.6 | 12.9 | 72.2 | 1.4×10^{-28} | 4.1×10^{-26} |
| CASPR2 | DRB1*11:01 | 9.4 | 4.6 | 19.3 | 2.0×10^{-8} | 5.7×10^{-6} |
| LGI1 | DRB1*07:01-DQA1*02:01-DQB1*02:02 | 5.2 | 3.2 | 8.6 | 4.7×10^{-11} | 2.3×10^{-9} |
| LGI1 | DRB1*07:01-DQA1*02:01-DQB1*03:03 | 3.1 | 1.7 | 5.5 | 4.4×10^{-4} | 2.1×10^{-2} |
| LGI1 | DPA1*02:01-DPB1*11:01 | 4.8 | 2.5 | 8.5 | 1.0×10^{-5} | 3.8×10^{-4} |
| LGI1 | C*06:02-B*57:01 | 3.6 | 1.9 | 6.2 | 1.3×10^{-4} | 8.8×10^{-3} |
| CASPR2 | DRB1*11:01-DQA1*05:01-DQB1*03:01 | 7.4 | 3.5 | 15.2 | 1.1×10^{-6} | 5.7×10^{-5} |

Corrected P-values indicate comparison between disease and healthy controls.

CASPR2-antibody-associated haplotype (Supplementary Table 4).

Discussion

This study is the first comparative HLA analysis of LGI1- and CASPR2-autoantibody mediated diseases, and shows marked and strikingly different HLA associations for these patients, at both allelic and haplotypic levels. Given the frequently overlapping clinical features in patients with LGI1 and CASPR2 antibodies, and their co-expression in VGKC complexes, these findings indicate that dichotomous predisposing HLA variants govern the generation of LGI1 versus CASPR2 antibodies. Furthermore, they strongly implicate T cells in disease initiation and the candidate HLA-binding peptide partners generated by our *in silico* data may help identify these interacting T cells.

While HLA-DRB1*07:01 and linked class II alleles, including the haplotype HLA-DRB1*07:01-DQA1*02:01-DQB1*02:02, showed very strong associations with LGI1-antibody patients, this was not observed among CASPR2-antibody patients in whom we found clear associations with HLA-DRB1*11:01 only. Among LGI1-antibody patients, DRB1*11:01 was observed at around healthy control rates, DRB4 was less frequently detected than DRB1*07:01, homozygosity for HLA-DRB1*07:01 was recognized, and other independent associations involved HLA class I alleles HLA-B*57:01 and HLA-C*06:02. Albeit limited by their intrinsic rarity, intriguingly, the three patients with both LGI1 and CASPR2 antibodies had yet another complement of HLA alleles. Perhaps this implicates further divergence in molecular mechanisms responsible for the generation of both auto-antibody specificities within an individual. However, the HLA associations do not appear to distinguish between

sub-phenotypes, outcomes or, in contrast to a previous observation, the presence of associated tumours (van Sonderen *et al.*, 2017). Also, the 9–27% frequencies of these HLA variants in healthy Caucasians are far higher than disease prevalence, implicating additional loci, environmental or stochastic influences in disease manifestation.

Furthermore, our data also provide several intriguing insights into the immunopathogenesis of these diseases. First, they extend the frequent HLA associations in IgG4-related diseases (Huijbers *et al.*, 2015), but here, exceptionally, with no DQ5 association. Second, the presence of dominant HLA class II associations implicates extracellular antigen processing and CD4 T cells in disease initiation (Trowsdale and Knight, 2013), but the LGI1 antibody class I associations found here, and HLA-B*44:03 and HLA-C*07:06 reported in seven patients previously (Kim *et al.*, 2017), are compatible with a role for intracellular antigen processing, including viruses and drugs. These class I differences between studies may be explained by ethnicity, sample size and relatively weak associations (Kim *et al.*, 2017; van Sonderen *et al.*, 2017). Indeed, this extended haplotype and the related complex linkage disequilibrium in this region of the genome warrant further analysis. Furthermore, our original hypothesis of adverse drug reaction-related HLA variants may relate to the linked adverse drug reaction-related class I and II HLA variants (HLA-DRB1*07:01, HLA-DQA1*02:01, HLA-B*57:01) (Yip *et al.*, 2015). These and future observations in patients with LGI1 antibodies may inform the genetic basis of more common adverse drug reactions. Third, the HLA similarities between tumour and non-tumour LGI1-antibody cases suggests the absence of a unique paraneoplastic signature, in contrast to Lambert-Eaton myasthenic syndrome (Wirtz *et al.*, 2005). Perhaps this implies tumours in patients with LGI1 antibodies largely reflect the age-matched background rate, rather than a distinct immune

mechanism, although a paucity of tumours classically associated with paraneoplastic neurological syndromes may limit our interpretation. Finally, our *in silico* predictions suggest that HLA-DQA1*02:01-DQB1*03:03 is unlikely to mediate presentation of LGI1-derived peptides, whereas the HLA-DQA1*05:01-DQB1*03:01 heterodimer may be implicated in the CASPR2-antibody phenotype. Also, the promiscuity of both CASPR2 and LGI1 peptides for some HLA variants, including HLA-DRB1*07:01 (Kim *et al.*, 2017), may explain why immunization with the same VGKC complexes may generate two distinct disease entities, and underlie the observed co-existence of both antibodies at rates far higher than expected by chance (Irani *et al.*, 2012). However, this *in silico* approach is inherently limited by the possibility that high affinity peptides are more effectively deleted through central tolerance. Nevertheless, taken together, the range of antigen-restricted peptides derived herein, and the relative HLA variant frequencies in disease versus control populations, generate hypothesis-driven approaches to expand disease-specific T cells *in vitro* and complement recent clinical and laboratory observations which strongly implicate T cell dependence of antibody-mediated diseases (Makuch *et al.*, 2018; Wilson *et al.*, 2018a, b).

In summary, the distinct HLA associations in patients with LGI1 and CASPR2 autoantibodies, together with differing clinical features relating to autoimmunity, support an immunological dissociation in generation of these clinically-overlapping autoantibody-mediated syndromes. The dominant class II HLA involvement combined with *in silico* predictions, offers potential to better understand the likely initiating T–B cell interactions. Further work should focus on the environmental factors that influence the presentation of peptides in genetically predisposed individuals.

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Conflicts of interest

S.R.I. and P.W. are co-applicants and receive royalties on patent application WO/2010/046716 entitled ‘Neurological Autoimmune Disorders’. The patent has been licensed to Euroimmun AG for the development of assays for LGI1 and other VGKC-complex antibodies. Other authors report no conflicts of interest.

Supplementary material

Supplementary material is available at *Brain* online.

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